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(54) Title: PROCESS FOR INSOLUBILIZING N-CARBOXYALKYL DERIVATIVES OF CHITOSAN

(57) Abstract

A method for insolubilizing films of N-carboxyalkyl derivatives of chitosan uses an annealing process to provide materials which can have varied degradation times. These materials can be used for various biomedical applications such as adhesion prevention. The annealing process heats the film for predetermined times at predetermined temperatures to vary the solubility of the film.

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PROCESS FOR INSOLUBILIZING N-CARBOXYALKYL DERIVATIVES OF CHITOSAN

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method of temporarily insolubilizing films and gels of N-carboxyalkyl derivatives of chitosan to provide materials which can have varied degradation times for various biomedical applications such as adhesion prevention. More particularly, the method relates to an annealing process in which the material is insolubilized by exposing film to temperatures between 50°C and 200°C for various lengths of time.

Description of the Prior Art

Chitin and chitosan (partially deacetylated chitin) are well known biocompatible materials whose preparation has been described in U.S. Patent 2,040,880, which issued on May 19, 1936. A derivative of chitosan, N,O-carboxymethyl chitosan, and its production has been described in U.S. Patent 4,619,995, which issued to E.R. Hayes in October of 1986. The preparation of another chitosan derivative, N-carboxybutyl chitosan, is described in U.S. Patent 4,835,265.

The uses of chitin, chitosan, and other polysaccharides in biomedical applications is most evident in wound dressings. Materials for use in wound dressing applications are disclosed in U.S. Patents 3,632,754, 4,532,134, 4,659,700, 4,572,906, 4,378,017, foreign patents GB 2026516, EP 0200574 and publications WO 86/00912 and WO 87/07618. Others have addressed the problem of adhesion prevention utilizing biodegradable materials. U.S. Patent 4,603,695, which issued August 5, 1986 to Ikada et al, discloses the use of an absorbable polyester polymer. Chitin and Chitosan can be absorbed by hydrolysis *in vivo*.

Co-pending application Serial No. 07/644,758, filed on January 24, 1991 and assigned to the assignee of the present invention, teaches the use of chitosan and derivatives thereof for adhesion prevention. The teachings of this application are incorporated herein by reference. None of these patents or patent applications,

however, teach a simple way of insolubilizing films and gels from derivatives of chitin for internal application to vary degradation times.

As described in co-pending application Serial No. 07/644,758, scar tissue results from the organization of fibrinous exudate on tissue surfaces due to the infliction of trauma or inflammation. Vital tissues such as blood vessels, or organs including the kidney, liver, and intestines are coated with mucous or serous membranes so that they can function independently of each other. Examples of the mucous or serous membranes are the body wall pleura and the organ pleura in the thoracic cavity and the parietal peritoneum and mesentery in the abdominal cavity, each protecting the corresponding organs. Surgical trauma or inflammation in those portions of the body coated with serous membranes could result in the build up of fibrinous exudate regardless of the size of the affected part. This ultimately causes the creation of organized fibrin many times referred to as scarring or adhesions. Such adhesions between these tissues may be observed in all tissues of the body, not just those mentioned above. Scarring between tissues can lead to severe pain, decreased function, and even permanent loss of motility.

Adhesions occur in the orthopedics field where conditions such as acute or chronic arthritis, suppurative, rheumatoid, gonorrheal, or tuberculous arthritis, or traumatic injuries at the joint, such as fracture or sprain, result in ankylotic diseases wherein the surface of the bones constituting the joint adhere to each other and thereby restrict the mobility of the joint. In addition, congenital radioulnar syntosis, wherein a spoke bone and an ulna adhere together, is difficult to remedy by a surgical operation, since the separated bones frequently re-adhere. Adhesions are also prominent in tendon surgery. In this instance, there is a general tendency towards adhesion between the tendon and the surrounding sheath or other surrounding tissue during an immobilization period following the operation.

More recently, there has been increased interest in the prevention of the "laminectomy membrane" which forms following spinal laminectomy procedures. The laminectomy membrane is a well organized mass of fibrinous tissue which replaces the bone that was removed at the laminectomy. This fibrinous mass binds the dura to the overlying muscles. This causes narrowing of the spinal canal which places pressure on the cauda equina or nerve roots. This scar tissue formation may require reoperation which is tedious and dangerous leading to the possibility of dural

tears and the damage to the emergent nerve roots resulting in motor weakness, sensory change, and painful paresthesia.

Numerous papers have been published on the various treatments to prevent scar tissue build up. Treatments such as liquid paraffin, camphor oil, chondroitin sulfate, and urea exhibit an insufficient effect since they function only temporarily. Other prophylactic treatments such as silicone membranes, gutta percha, or poly (tetrafluoroethylene) membranes have been used to serve as barriers to adhesion formation. However, these materials are inert and will, therefore, remain in the body and many times be recognized as a foreign body. Therefore, a second operation may be necessary to remove the barrier material.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a process for producing modified N-carboxyalkyl derivatives of chitosan for the prevention of fibrinous exudate build-up, which degrades *in vivo* in various predetermined time periods.

It is a further object of the invention to provide a simple annealing process for insolubilizing N-carboxyalkyl derivatives of chitosan.

Accordingly, these and related objects are achieved by a process for insolubilizing films of N-carboxyalkyl derivatives of chitosan. The N-carboxyalkyl derivative of chitosan is dissolved in a neutral pH aqueous solution or a slightly acidic solution. The solution is then cast and dried to form a thin clear film. Alternatively, the solution is poured into a mold (for example a petri dish) and lyophilized to form a sponge-type film with varying dimensions depending on the mold, the solution concentration, and the solution volume. The resulting films are insolubilized by an annealing process in which they are exposed to temperatures between 50°C and 200°C for various periods of time depending on the desired length of insolubilization time, and ultimately bioresorption time.

These and other objects and advantages of the present invention will become apparent from the following detailed description which discloses several embodiments of the invention. It is to be understood that the examples used are for the purposes of illustration only, and not as a definition of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The biodegradable polymer films to be used to inhibit fibrin formation and organization are materials which will eventually revert to the gel or solution state and ultimately be resorbed and metabolized by the body. As taught in co-pending application Serial Number 07/644,758, these materials include amino N-carboxyalkyl derivatives of chitosan. Chitosan is a partially deacetylated chitin defined for the purposes herein as being greater than 50% deacetylated.

The N-carboxyalkyl derivatives used in the present invention are water soluble polymers which have not been crosslinked to form insoluble materials. Specific examples of these materials are N-carboxymethyl chitosan, N-carboxybutyl chitosan, N,O carboxymethyl chitosan, and N,O-carboxybutyl chitosan, N-carboxyethyl chitosan, N,O-carboxyethyl chitosan, N,O-carboxypropyl chitosan, and N-carboxypropyl chitosan. However, these materials can be temporarily insolubilized to form substances which will begin to degrade in a period of from 2-5 days to up to one year *in vitro*. It has been found that exposure of films of these materials to heat from 50°C to 200°C for various time periods varies the insolubilization time and ultimately the bioresorption time. In general, the higher the temperature of exposure and the longer the time of exposure, the greater the time to solubilize the film. In order to vary the degradation time, the temperature and the length of time of heat exposure are varied from 50° - 200°C anywhere from 20 minutes to 24 hours.

These materials are prepared from natural products or by fermentation methods as described in U.S. Patents 4,835,265 and 4,619,995. The molecular weight of the biodegradable N-carboxyalkyl derivatives of chitosan for use in the present invention preferably range from 1,000 daltons to 3,000,000 daltons.

In the preferred embodiment, these polymers would be in the form of a film, sponge, or woven sheet which will break down into visco-elastic materials. Examples of these would be the use of N,O-carboxymethyl chitosan or N-carboxybutyl chitosan films insolubilized by annealing with heat as described herein. While the exact mechanism which causes the insolubilization of the materials is unclear, it is postulated that either a dehydration mechanism, or a crystallization mechanism or a combination of the two is causing this phenomena. Additives such as anti-thrombogenic materials may be added to the films before insolubilizing.

As described in co-pending application 07/644,758, it is also possible to form these materials into a viscous gel for injection into the affected location to prevent fibrinous buildup. This gel could be used for applications where a shorter *in vivo* residence time is desired and/or the location would best be suited for prevention of adhesions with a material in this form. The gels may be formed by placing the insolubilized films in a sterile aqueous media and heating the solution to increased temperatures until the film hydrates and swells to form a gelatinous mass.

Again, as with the film, the degree of insolubilization of the viscous gels can be varied by varying the temperature of the aqueous solution and the extent of time the film is allowed to remain in the solution.

The present invention has the added advantage that the insolubilization step can be done during the heat or steam sterilization of the device, if desired.

The invention will now be described in further detail with reference being made to the following examples. It should, however, be recognized that the examples are given as being illustrative of the present invention and are not intended to define the spirit and spirit thereof.

Example 1

One gram of medical grade N,O-carboxymethyl chitosan (NOVA Chem, Nova Scotia) was dissolved in 100 cc of purified water. The resulting 1% solution was then filtered through a series of cellulose membranes (12, 8, 0.45 micron) to remove all insoluble matter. The solution was then recirculated over and through a 0.5 micron tangential flow membrane which was specially treated with polypeptides specific for removal of pyrogen and other hydrophobic impurities (Catalog number 4200 - AlerChek, Portland, ME) for a period of approximately one hour on tangential flow ultra-filtration device (Filtron - Norwood, MA). The resulting solution was then rendered free of all low molecular weight impurities by extensive dialysis with water via a 300K molecular weight cutoff membrane (Filtron - Norwood, MA) on the same ultra-filtration device.

Films were then formed from this solution by both casting (allowing evaporation) on a non-stick surface such as a glass petri dish, or a piece of mylar film and by freeze drying by adding 300 ml of the solution to a 90 mm diameter disposable petri dish and lyophilized in a tray dryer for 72 hours at -50°C. The resulting films (either cast or freeze dried) were then insolubilized by placing the

film into an oven at 121°C for 20 minutes (correlating to a standard autoclave cycle). Both films were placed in aqueous media and dissolved within 5 days. Based on the dissolution which did occur, the film would dissolve in approximately two months.

Example 2

Three films were prepared as described above in Example 1 by casting in a petri dish. Each was exposed to a different temperature for a period of 30 minutes. The first was heated at 76°C, the second at 121°C and the third at 168°C. One film was cast and not treated. This served as a control.

The four films were placed in 5 cc of PBS and placed in an incubator at 37°C. At various times, the samples were removed and observed visually for solubility. Following this the incubation medium was removed from the dish and analyzed for soluble product via size exclusion chromatography. The control film was completely solubilized within one day. The film at 76°C indicated complete solubility between 1-2 days. The 121°C treated film indicated complete solubility at 4-5 days, while the film treated at 168°C indicated no evidence of solubility up to 14 days.

Example 3

Three films were prepared as described above in Example 1 with the exception that the temperature of annealing was kept constant at 121°C and the exposure time was varied between 15 minutes and 23 hours. Again, a non-treated film served as a control. All films were evaluated as mentioned above.

The film treated at 15 minutes indicated complete solubility in 2-3 days, the film treated for 60 minutes indicated solubility through 14 days (complete solubility had not yet been attained) while the film treated at 2 hours indicated no solubility in an excess of 14 days.

Thus the degradation of the films *in vivo* could be lengthened by increasing the annealing time and temperature. For a typical orthopedic application, a film which would degrade within 14 days is desirable and may be attained by annealing at 121°C for about 60 minutes.

While several examples of the present invention have been described, it is obvious that many changes and modifications may be made thereunto, without departing from the spirit and scope of the invention.

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CLAIMS:

1. A method for insolubilizing films made from N-carboxyalkyl derivatives of chitosan by heating the films at a temperature between 50°C and 200°C for a predetermined time.

2. The method as set forth in claim 1 wherein the derivative of N-carboxyalkyl chitosan is N,O-carboxymethyl chitosan.

3. The method as set forth in claim 1 wherein the derivative of N-carboxyalkyl chitosan is N-carboxymethyl chitosan.

4. The method as set forth in claim 1 wherein the derivative of N-carboxyalkyl chitosan is N,O-carboxyethyl chitosan.

5. The method as set forth in claim 1 wherein the derivative of N-carboxyalkyl chitosan is N-carboxyethyl chitosan.

6. The method as set forth in claim 1 wherein the derivative of N-carboxyalkyl chitosan is N,O-carboxybutyl chitosan.

7. The method as set forth in claim 1 wherein the derivative of N-carboxyalkyl chitosan is N-carboxybutyl chitosan.

8. The method as set forth in claim 1 wherein the derivative of N-carboxyalkyl chitosan is sulfated N carboxyalkyl derivatives of chitosan.

9. The method as set forth in claim 1 wherein the film is formed by casting an aqueous solution of said N-carboxyalkyl derivatives of chitosan on a flat surface and evaporating the aqueous media.

10. The method as set forth in claim 1 wherein said film is formed by placing an aqueous solution of said N-carboxyalkyl derivatives of chitosan in a mold and lyophilizing.

11. The method as set forth in claim 1 wherein the film includes an anti-thrombogenic agent.

12. The method as set forth in claim 1 wherein the film is insolubilized during the autoclave sterilization of the device at a temperature of greater than 120°C for a standard autoclave cycle.

13. The method as set forth in claim 1 wherein the predetermined time ranges from 10 minutes to 2 hours.

14. A method for preventing adhesions between soft internal body tissues comprising the steps of:

preparing an aqueous solution of N-carboxyalkyl derivatives of chitosan;

forming a film from said aqueous solution by removing the water therefrom;

insolubilizing said film by heating the film at a temperature of at least 76°C for a minimum of fifteen minutes; and

placing the film between said tissues after being insolubilized.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 92/10417

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C08B 37/00, C08J 5/18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C08B, C08L, C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CLAIMS, WPI, CAS ON LINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, Volume 115, No 20, 18 November 1991 (18.11.91), (Columbus, Ohio, USA), page 145, THE ABSTRACT No 210574j, JP, A, 3143901, (Nishiyama, Masashi et al) 19 June 1991 (19.06.91), the whole abstract ---	1-9
X	Chemical Abstracts, Volume 100, No 22, 28 May 1984 (28.05.84), (Columbus, Ohio, USA), Ogawa, Kozo et al, "A new polymorph of chitosan", page 667, THE ABSTRACT No 175178z, Macromolecules 1984, 17 (4), 973-975, (e), the whole abstract ---	1-8

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search	Date of mailing of the international search report
2 March 1993	25.03.93
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 92/10417

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, Volume 107, No 4, 27 July 1987 (27.07.87), (Columbus, Ohio, USA), Ban, Seiji et al, "Release of dimethacrylate monomers into water", page 319, THE ABSTRACT No 28320f, Aichi Gakuin daigaku Shigakkaishi 1986, 24 (3), 383-389, (e), the whole abstract	1
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A	US, A, 4619995 (E R HAYES), 28 October 1986 (28.10.86), abstract, claims	1-14
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A	EP, A2, 0426368 (PFIZER HOSPITAL PRODUCTS GROUP, INC.), 8 May 1991 (08.05.91), page 2, line 50 - page 3, line 9, claims 1-4, 13-17	1-14
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INTERNATIONAL SEARCH REPORT

Information on patent family members

29/01/93

International application No.
PCT/US 92/10417

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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US-A- 4619995	28/10/86	AU-B- 591713	14/12/89
		AU-A- 6366786	14/04/88
		EP-A,B- 0265561	04/05/88
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		JP-A- 63110201	14/05/88
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		CA-A- 2028709	01/05/91
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